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Integration of nuclear and mitochondrial gene sequences and morphology reveals unexpected diversity in the forest cobra (*Naja melanoleuca*) species complex in Central and West Africa (Serpentes: Elapidae)

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Abstract

Cobras are among the most widely known venomous snakes, and yet their taxonomy remains incompletely understood, particularly in Africa. Here, we use a combination of mitochondrial and nuclear gene sequences and morphological data to diagnose species limits within the African forest cobra, *Naja* (*Boulengerina*) *melanoleuca*. Mitochondrial DNA sequences reveal deep divergences within this taxon. Congruent patterns of variation in mtDNA, nuclear genes and morphology support the recognition of five separate species, confirming the species status of *N. subfulva* and *N. peroescobari*, and revealing two previously unnamed West African species, which are described as new: *Naja (Boulengerina) guineensis* **sp. nov.** Broadley, Trape, Chirio, Ineich & Wüster, from the Upper Guinea forest of West Africa, and *Naja (Boulengerina) savannula* **sp. nov.** Broadley, Trape, Chirio & Wüster, a banded form from the savanna-forest mosaic of the Guinea and Sudanian savannas of West Africa. The discovery of cryptic diversity in this iconic group highlights our limited understanding of tropical African biodiversity, hindering our ability to conserve it effectively.

Key words: Integrative taxonomy, Africa, *Naja melanoleuca*, *Naja guineensis* **sp. nov.**, *Naja savannula* **sp. nov.**, Elapidae, systematics

Introduction

In recent decades, the availability of increasingly practical and affordable molecular methods has revolutionised both our concept of what a species is (De Queiroz, 1998, 2007), and the way species are discovered and delimited. Prior to the 1960s, species were delimited solely on the basis of morphological differences. Since the 1990s, DNA

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sequence information has assumed a pivotal role in species delimitation, and the process of species discovery has increasingly focussed on the identification of historical lineages rather than phenotypically distinct or reproductively isolated entities. For many years, mitochondrial DNA (mtDNA) has been the mainstay of the phylogeographic approach to species delimitation (Avise, 2000), due in large part to its rapid rate of evolution, which provides a strong phylogenetic signal even for recent divergences. However, due to the matrilineal, non-recombining mode of inheritance of mtDNA, this marker on its own can be inadequate for the delimitation of species boundaries, due to effects such as male-mediated gene flow crossing boundaries between mtDNA haplotype clades (Thorpe & Richard, 2001; Ogden & Thorpe, 2002; Thorpe *et al.*, 2010), introgressive hybridisation (Babik *et al.*, 2005), and its inability to reveal intergradation on its own.

The inadequacies of mitochondrial DNA on its own have increasingly led to integrative approaches to species delimitation (Padial *et al.*, 2010), whereby multiple approaches are combined to define species limits. For instance, a combination of morphological variation and mtDNA phylogeography has been used successfully to delimit species (e.g., Wüster & Broadley, 2003, 2007; Malhotra *et al.*, 2011a) as well as to question them (Puorto *et al.*, 2001).

More recently, nuclear sequences have become increasingly widely used in phylogeographic analyses and to test species boundaries as part of an integrative approach to taxonomy. Since nuclear loci are not part of a single linkage unit with mtDNA, they provide an independent test of species boundaries, although interpretation and use is complicated by several factors (Hare, 2001). One of the difficulties in using nuclear genes to reconstruct phylogenies at low taxonomic levels is the fact that most nuclear genes have considerably lower rates of evolution than most mitochondrial genes, so that they often carry little phylogenetic signal in analyses around the species level, and their greater effective population size leads to slower lineage sorting and thus greater difficulty in identifying recently isolated lineages. Recent efforts have identified several genes that evolve at faster rates than more traditional nuclear genes (Townsend *et al.*, 2008), making the use of single copy nuclear genes for species delimitation a more realistic possibility.

Understanding species limits is of practical as well as academic importance. Conservation requires the recognition and identification of threatened taxa (May, 1990; Hekkala *et al.*, 2011). Moreover, for medically important taxa such as venomous snakes, a sound taxonomic framework provides the essential underpinnings for research into venom variation and the preparation of effective antivenoms (Fry *et al.*, 2003; Williams *et al.*, 2011) as well as for studies on the causes and correlates of venom variation (Barlow *et al.*, 2009; Casewell *et al.*, 2013, 2014).

Among venomous snakes, cobras (*Naja*) have been the subject of extensive revision, bringing the number of recognised species from five (Department of the Navy, 1965) to a current total of 29 species (Wüster *et al.*, 2007; Wallach *et al.*, 2009; Ceríaco *et al.*, 2017). Wüster *et al.* (2007) identified four major lineages within the genus *Naja*, the Asian cobras, the African spitting cobras, the Cape/Egyptian cobra group and a mostly Central African group containing the forest cobra *Naja melanoleuca* as well as the water cobras (*Naja annulata* and *N. christyi*) and the burrowing cobra (*N. multifasciata*). The water and burrowing cobras were previously recognised as distinct genera, *Boulengerina* and *Paranaja*, respectively. Wüster *et al.* (2007) and Wallach *et al.* (2009) emphasised the need to conserve the nomenclature stability of the genus *Naja*, a group of iconic and medically important taxa. Wallach *et al.* (2009) recognised the four major clades identified by Wüster *et al.* (2007) as four subgenera within *Naja*: *Naja*, *Afronaja*, *Uraeus* and *Boulengerina*. This arrangement recognises the major monophyletic groups contained within *Naja* while retaining nomenclatural stability. However, Wallach *et al.* (2014), and subsequently Ceríaco *et al.* (2016) recognised these subgenera at full generic level without providing any explanation or justification for this act. Since this not only undermines the nomenclatural stability of a long-established genus but also leads to the loss of a tier of phylogenetic information conveyed by the use of the genus-subgenus combination, we retain *Naja* as a single genus *sensu* Wallach *et al.* (2009) and recommend against raising the subgenera to genus level.

The species limits within three of the four subgenera have been extensively revised: *Naja* now contains 11 Asian species (Wüster & Thorpe, 1991; Wüster, 1996; Slowinski & Wüster, 2000), *Afronaja* seven African spitting cobras (Wüster *et al.*, 2007), and *Uraeus* six African non-spitting, open-formation species (Broadley & Wüster, 2004; Trape *et al.*, 2009). In contrast, species limits within the remaining subgenus, the morphologically and ecologically diverse African forest subgenus *Boulengerina*, have remained largely understudied. Four species have been widely recognised for decades. Three are relatively infrequently encountered and appear to have specialised

life histories: two water cobras, *Naja (Boulengerina) annulata* and *N. (B.) christyi* and the so-called burrowing cobra, *N. (B.) multifasciata*, previously *Paranaja multifasciata*.

The fourth long-recognised species of the subgenus is the forest cobra, *Naja (Boulengerina) melanoleuca* Hallowell, 1857, which is widespread and frequently encountered in all forested parts of tropical Africa as well as adjoining forest-savanna mosaic formations. *Naja melanoleuca* was originally described from Gabon as a variety of *Naja haje* (Linnaeus), and was not recognised as a full species until late in the 19th century (Matschie, 1893). The species has since received little taxonomic interest. Geographic variation in pattern and, to a lesser extent, scalation has been noted (e.g., Boulenger, 1896; Laurent, 1955, 1956, 1973; Broadley, 1968; Spawls & Branch, 1995; Spawls *et al.*, 2002, 2018). Laurent (1955) described *Naja melanoleuca subfulva* from South Kivu Province of Democratic Republic of the Congo (DRC), with paratypes from Rwanda and Burundi. Broadley (1968), in a brief review of the African species of *Naja,* placed *subfulva* in the synonymy of *N. melanoleuca,* noting that specimens from Uganda agreed with *melanoleuca* in colouration, but with *subfulva* in ventral counts. Recognition of the name *subfulva* has been sporadic (but see Laurent, 1973), but Chirio & Ineich (2006) and Ceríaco *et al.* (2017) recognised it as a separate species, the latter with supporting molecular evidence. Stucki-Stirn (1979) described *N. m. aurata* from the grassfields of western Cameroon, but this subspecies was not recognised by subsequent authors. Finally, Ceríaco *et al.* (2017) found molecular and morphological evidence to describe the São Tomé population as a new, endemic species, *Naja peroescobari*.

Preliminary work by one of us (WW) and Ceríaco *et al.* (2017) revealed deep phylogeographic splits and extensive mtDNA diversity within *N. melanoleuca* in West and Central Africa, suggestive of the presence of multiple species, which led to the present study. Our aim is to use an integrative taxonomic approach and a combination of nuclear and mitochondrial gene sequences as well as morphology to delimit the species within the *N. melanoleuca* complex. We follow the basic approach of Padial *et al.* (2010): mitochondrial DNA will be used to identify candidate species within the complex, and these will then be further tested with nuclear DNA sequences and morphological data to either confirm or reject their status as full species. Here, we recognise as candidate species sets of populations separated by mitochondrial p-distances of 0.035 or greater, corresponding to those observed between sister species in other *Naja* (Slowinski & Wüster, 2000; Wüster & Broadley, 2003, 2007; Wüster *et al.*, 2007; Trape *et al.*, 2009). We then recognise them as full species if they also display at least one of the following: diagnostic character states; clear separation in multivariate morphometric analyses; lack of shared single-copy nuclear haplotypes; evidence of distinctiveness in Bayesian species delimitation that is robust to different priors; and distinctness in multilocus genetic distance.

Materials and methods

Molecular methods. We obtained tissue samples (scale clippings, blood, shed skins, dermal tissue or liver) from 71 individuals, providing broad geographic sampling across the full distribution of the *N. melanoleuca* complex in Africa. Details of samples and vouchers are given in Appendix 1. Whole genomic DNA was extracted using a Qiagen DNeasy™ Tissue Kit (catalogue no. 69506), following the manufacturer's instructions, except for blood samples, where PBS buffer was not used and 200µl of blood in Tris-EDTA buffer was added to 20µl proteinase K. Where shed skins were used, the samples were left to lyse overnight. The amount of DNA present in each extraction was then estimated using a Nanodrop™ spectrophotometer. Fragments of the mitochondrial genes NADH dehydrogenase subunit 4 (ND4) and cytochrome *b* (cyt *b*), and two single copy nuclear genes, the prolactin receptor gene (PRLR) and ubinuclein 1 (UBN1) (Townsend *et al.*, 2008), were PCR-amplified and sequenced for all individuals. Primer combinations are given in Table 1.

The PCR reaction mix consisted of 0.3 µl of each primer, 0.8–2.0 µl template DNA and 9.6 µl ABgene® 1.1x ReddyMix™ (1.25 units Thermoprime Plus DNA Polymerase, 75mM Tris-HCL (pH 8.8 at 25ºC), 20mM $(NH_4)_2SO_4$, 2.0mM $MgCl_2$, 0.01% (v/v) Tween® 20, 0.2mM of each dNTP and a red dye and precipitant for gel electrophoresis (ABgene 2005). 0.8 µl of template DNA was used for ND4 and cyt *b*, whereas 2.0 µl of template was used for PRLR and UBN1.

The thermocycling regimes involved an initial denaturation at 94°C for 2 minutes (m); 35–40 cycles of: 30 seconds (s) denaturation at 94°C, 30s annealing at 47°C (cyt *b*), 54°C (ND4) or 45°C, 1m extension at 72°C; and a final extension for 5m at 72°C. All PCR products were cleaned by adding 1µl shrimp alkaline phosphate (SAP) and

0.1µl exonuclease 1 (EXO) to each sample. Samples were then run in a thermal cycler for an initial incubation period of 37°C for 1 hour, 80°C for 15m, followed by a termination period of 4°C for 30s. Direct sequencing was carried out by Macrogen Inc. (dna.macrogen.com) using the same forward primers used in PCRs for mitochondrial fragments and both forward and reverse primers for nuclear loci.

TABLE 1. PCR and sequencing primer sequences and sources.

Gene	Primer	Primer sequence	Reference
ND4	ND4	5'– CACCTATGACTACCAAAAGCTCATGTAGAAGC – 3'	Arévalo *et al.* (1994)
	H127	5'– TTCTATCACTTGGATTTGCACCA – 3'	Wüster *et al.* (2008)
Cyt *b*	Gludg	5'– TGACTTGAARAACCAYCGTTG – 3'	Palumbi (1996)
	H16064 or	5'– CTTTGGTTTACAAGAACAATGCTTTA – 3'	Burbrink *et al.* (2000)
	ATRCB3	5' – TGAGAAGTTTTCYGGGTCRTT – 3'	Harvey *et al.* (2000)
PRLR	PRLR F1	5'– GACARYGARGACCAGCAACTRATGCC – 3'	Townsend *et al.* (2008)
	PRLR r3	5'– GACYTTGTGRACTTCYACRTAATCCAT – 3'	Townsend *et al.* (2008)
UBN1	BaUBN F	5'– ATTGGCCACTCCTTGTGTTC' – 3'	Casewell *et al.* (2011)
	BaUBN R	5'– ATTGGCCACTCCTTGTGTTC – 3'	Casewell *et al.* (2011)

Sequence chromatograms were proofread and aligned using the software CodonCode Aligner Version 3.5.6 (www.codoncode.com). All sequences were translated to check that no frame shift mutations or unexpected stop codons were present. Heterozygous positions in nuclear sequences were identified by a combination of visual inspection for double peaks and typically low quality Phred scores (Ewing *et al.*, 1998) for the bases surrounding a heterozygous position. Individual allele sequences (haplotypes) were estimated from diploid nuclear loci using the software PHASE v. 2.1.1 (Stephens *et al.*, 2001; Stephens & Scheet, 2005) over 5000 iterations with a burn-in of 500 and a thinning interval of 10, after preparation of the sequence data using SEQPHASE (Flot, 2010). PHASE was run three times to confirm burn-in and convergence across multiple runs.

A mitochondrial DNA phylogeny was generated by maximum likelihood (ML) analysis using the GTR+I+G model identified as optimal in MEGA 6.06 (Tamura *et al.*, 2013) and a neighbour-joining starting tree, and branch support was assessed by means of 1000 bootstrap replicates. Sequences of four species of *Naja* representing the other subgenera were included to root the tree: *N. (Naja) naja*, *N. (Uraeus) senegalensis*, *N. (Afronaja) ashei* and *N. (Afronaja) nubiae*. Deep mitochondrial haplotype clades were then identified as candidate species to be tested in subsequent analyses.

Patterns of haplotype sharing in the two nuclear loci were visualised using the programme Network 4.6.1.0 (Fluxus Technology Ltd. – www.fluxus-engineering.com), and networks drawn in the associated software Network Publisher 1.3.0.0. We obtained an overall measure of nuclear genetic distance between individuals using the program POFAD v.1.03 (Joly & Bruneau, 2006). Allele distance matrices were generated for each nuclear locus under the Kimura two-parameter model (K2P; Kimura, 1980) in PAUP*4.0b10 (Swofford, 2002). A matrix of standardised inter-individual distances across both loci was then calculated in POFAD. This was then input into the software MVSP (Kovach Computing Services, Pentraeth, UK; www.kovcomp.co.uk), and subjected to a principal coordinates analysis to generate a graphical representation of the inter-individual distances.

Bayesian species delimitation was implemented using the reversible-jump Markov Chain Monte Carlo (rjMCMC) method of Yang & Rannala (2010), using the software BPP Version 3 (Rannala & Yang, 2003; Yang & Rannala, 2014), which incorporates species tree uncertainty. This method accommodates the species phylogeny as well as lineage sorting due to ancestral polymorphism while using a nearest-neighbour interchange (NNI) to alter the species tree.

In any Bayesian analysis, the results are likely to be critically dependent on the priors. The key priors for Bayesian species delimitation are the ancestral population size (θ) and root age (τ_0). Both can affect the posterior probabilities for models (Yang & Rannala, 2010). Since neither of these values can be inferred for the *Naja melanoleuca* group, we instead assessed the effects of different combinations of priors on the robustness of species inferences. We therefore varied values of θ and τ_0 to simulate different ancestral population sizes and ages of nodes (Leaché & Fujita, 2010). We considered the four possible combinations of extremes of small or large ancestral

population size and shallow or deep divergences. Large and small ancestral population size estimates were modelled as $\theta \sim G(1,10)$ and $\theta \sim G(2,2000)$, respectively, and deep and shallow divergences as $\tau_0 \sim G(1,10)$ and $\tau_0 \sim G(2,2000)$ respectively (Leaché & Fujita, 2010). Priors assuming large ancestral population sizes and shallow divergences are likely to be more conservative in terms of numbers of species estimated. This applies particularly since most taxa of the *N. melanoleuca* complex occupy large areas and there is no specific reason for assuming a small founder stock (except in the case of *Naja peroescobari* on São Tomé Island). All BPP3 runs involved a sample size of 100,000, a burnin of 20,000 and a sampling frequency of 2, and were run in duplicate to ensure burnin and consistency of results.

Morphology. Based on mtDNA data showing deeply divergent clades in the complex in Central and West Africa, we examined all available material from west of the Rift Valley and surrounding areas. Further south and east, considerable variation in colour pattern and scalation is not accompanied by deep mitochondrial divergence, but is likely to confound morphological discrimination between distinct lineages in West and Central Africa. We therefore excluded material from southern and parts of eastern Africa from our morphological analyses, pending additional work.

The candidate species identified in this species complex show no diagnostic features in their head scalation, and there is limited interspecific variation in classical scale counts. Dorsal colouration and the patterns of dark crossbars on the venter provide useful diagnostic characters, but these may be confounded by ontogenetic changes. The ventral pattern of the *N. melanoleuca* complex can be summarised as follows: the anterior venter, throat and chin are predominantly white, with one or several darker crossbands. Following on from this banded anterior ventral region, in most specimens the venter then darkens gradually towards the vent, becoming uniformly dark brown or black more or less rapidly along the posterior half of the venter. We recorded the pattern of ventral banding by noting the ventral scale number of all dark pigmented ventral scales. The ventral bands were divided into main bands and accessory bands. In the *N. melanoleuca* complex, the main bands are generally well defined, wide (4 or more ventral scales) and in similar positions across most specimens. Additional narrower (usually covering 1–2 ventrals, and not always the entire scale) accessory bands may be present between some of the main bands and under the anterior throat, and the number of these was recorded as well. Finally, we recorded the last ventral of the posteriormost discrete dark main band as a proxy for the posterior extent of well-defined banding along the ventral side. The characters retained for the final analysis are listed in Table 2. The initial separation of taxa was based on the analysis of meristic data for most specimens available in museum collections, collected by DGB over a period of 40 years from personal inspection, literature records, correspondence and relevant data supplied by Barry Hughes.

TABLE 2. Morphological characters used in analyses of the *Naja melanoleuca* complex

Number	Character
1	Midbody scale rows: number of dorsal scale rows at midbody
2	Ventral scales, the first being defined as the first scale wider than long behind the gulars
3	Subcaudal scales, excluding the terminal conical scale
4	Number of discrete dark main bands on the ventral side
5	Number of discrete accessory dark bands across the ventral surface
6	Total number of discrete dark bands across ventral surface
7	Position of first ventral scale involved in the first main dark band
8	Last ventral scale of the first main dark band
9	Width in ventral scales of the first dark band
10	Position of the last dark ventral of the last discrete main dark band

Our final analysis used 605 specimens of the *N. melanoleuca* complex. Collection acronyms follow Leviton *et al.* 1985, with the following additions: E3M= University of Ghana, Legon (B. Hughes); EBM = Estação Biologia Maritima, Inhaca Island, Mozambique; JPT = Zinave National Park, Mozambique (J.P. Tello); KMH = University of Dar es Salaam, Tanzania (K.M. Howell). The specimens examined in the Museu Bocage, Lisbon (MBL) were subsequently destroyed by fire.

Our principal method of analysis was Canonical Variate Analysis (CVA), which requires the grouping of specimens into operational taxonomic units (OTUs). OTUs were initially defined based on mitochondrial candidate species; these were then further subdivided based on collecting gaps within their distributions. Since the mitochondrial and nuclear DNA data suggested sympatry between different candidate species, the proposed OTUs were initially checked for homogeneity by means of principal components analyses (PCA) run on standardised data, and the assignment of specimens to OTUs corrected based on the results. Once OTUs were defined (Table 3), a CVA was run on characters 1–5, 7, 9 and 10 (since characters 6 and 8 duplicated information contained in the others). The final analysis was run without OTU 13 as the sample size for CS5-*peroescobari* was too small to be useful for canonical variates analysis.

TABLE 3. OTUs defined for canonical variates analysis of the *Naja melanoleuca* complex. DRC = Democratic Republic of Congo.

OTU number	Candidate species	Distribution
1	CS4-Wblack	Guinea, Sierra Leone, Liberia
2	CS4-Wblack	Ivory Coast, Ghana, Togo
3	CS1-*melanoleuca*	Western Nigeria
4	CS1-*melanoleuca*	Eastern Nigeria, Cameroon, Gabon
5	CS1-*melanoleuca*	Central African Republic (CAR), northern Republic of Congo (RoC), northern Democratic Republic of the Congo (DRC)
6	CS1-*melanoleuca*	southern RoC, southwestern DRC
7	CS1-*melanoleuca*	eastern DRC
8	CS3-Wbanded	Senegal, Guinea, Mali
9	CS3-Wbanded	Ivory Coast, Ghana, Benin, Nigeria, Cameroon
10	CS2-*subfulva*	Cameroon, CAR, northern RoC
11	CS2-*subfulva*	northeastern DRC, Uganda, Kenya
12	CS2-*subfulva*	southeastern DRC, Burundi
13	CS5-*peroescobari*	São Tomé

Results

Molecular data. We aligned 716 b.p. of cytochrome *b*, 660 b.p. of ND4, 510 b.p. of nuclear PRLR and 436 b.p. of nuclear UBN1 sequence. Of the 1376 mitochondrial base pair positions, 449 are variable and 321 informative under the parsimony criterion (314 variable and 259 parsimony informative when outgroup sequences are excluded). Corresponding figures for PRLR and UBN1 (ingroup only) are 20 variable and 14 parsimony-informative and 13 variable and 11 parsimony-informative positions, respectively. All sequences have been deposited with GenBank (Accession numbers GQ359493, GQ359497, GQ359575, GQ359579, GQ387080, GQ387109, MH337375–MH337634).

Mitochondrial phylogeny. The phylogenetic tree derived from the mitochondrial data (Fig. 1) reveals five major clades, here considered as candidate species (CS) (Padial *et al.*, 2010), differing by average p-distances of 0.065 to 0.108 across cytochrome *b* and ND4:

CS1-*melanoleuca*: A Central African forest form extending from western Cameroon across the Congo Basin to northwestern Angola and Lake Kivu, including topotypical populations of the nominate form *N. melanoleuca melanoleuca* from Gabon.

CS2-*subfulva*: A widespread eastern form extending from western Cameroon to coastal Kenya and south to South Africa (northern KwaZulu-Natal) as well as across the Congo Basin, including topotypical populations of *Naja melanoleuca subfulva*.

CS3-Wbanded: A West African form with obvious dorsal banding extending from Senegal to Cameroon in the West African savanna belt.

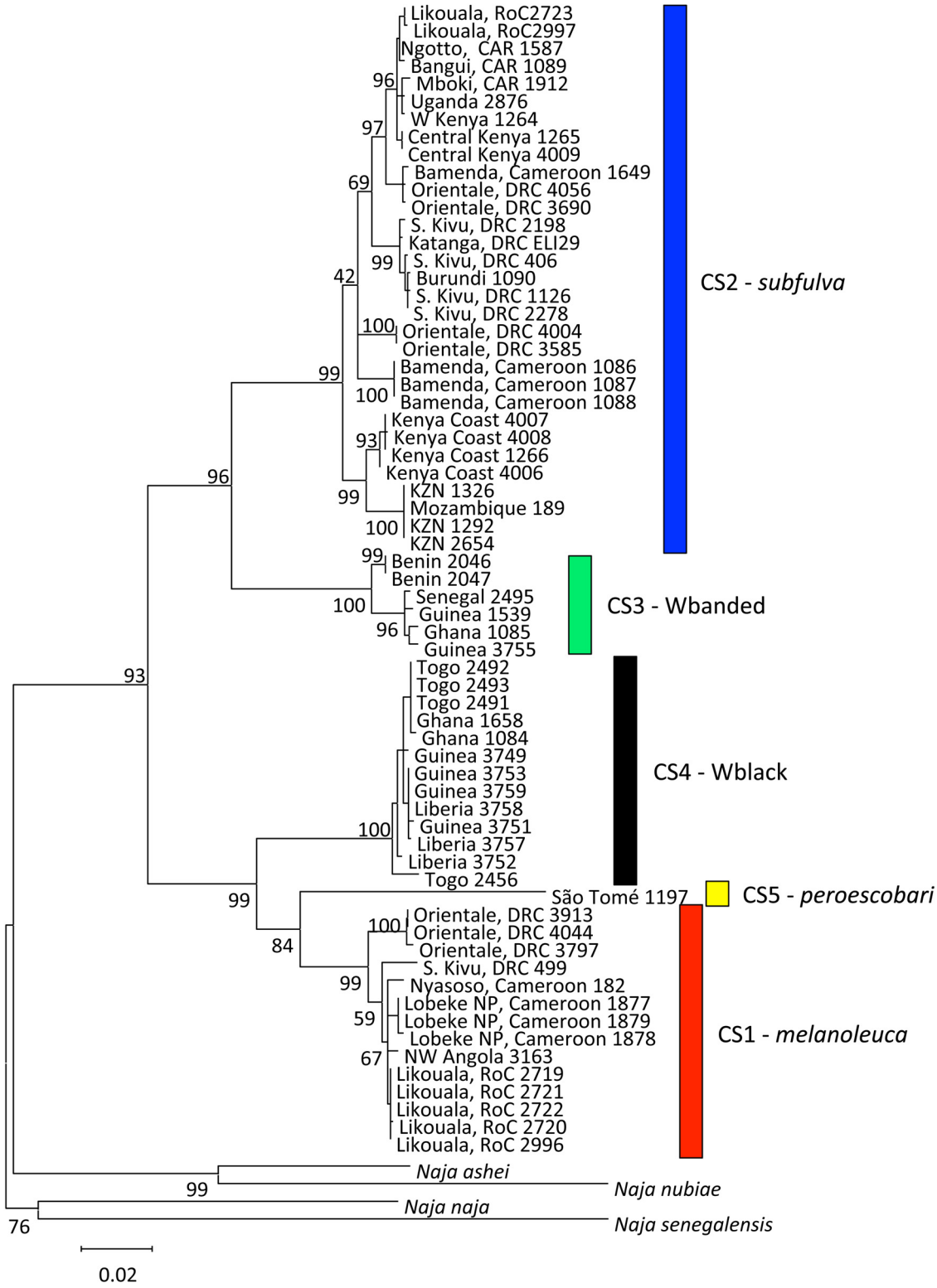

FIGURE 1. Maximum likelihood mitochondrial phylogeny of the *Naja melanoleuca* complex. Node support values indicate % bootstrap support; support values for the most distal nodes not shown. Country abbreviations: CAR = Central African Republic, DRC = Democratic Republic of Congo, KZN = KwaZulu-Natal Province, South Africa, RoC = Republic of Congo. Mitochondrial candidate species (CS) are shown in the same colours as in Figures 2–4. For specimen information see Appendix 1.

CS4-Wblack: A frequently melanistic West African forest form extending from Guinea to Togo.
CS5-*peroescobari*, endemic to the island of São Tomé in the Gulf of Guinea.

Mean pairwise mitochondrial sequence divergences among these candidate species are shown in Table 4.

TABLE 4. Mean between-group p-distances across 1326 b.p. of mitochondrial cyt *b* and ND4 sequence.

	CS1-*melanoleuca*	CS2-*subfulva*	CS3-Wbanded	CS4-Wblack
CS1-*melanoleuca*	–			
CS2-*subfulva*	0.09020	–		
CS3-Wbanded	0.09210	0.06747	–	
CS4-Wblack	0.06458	0.09452	0.09238	–
CS5-*peroescobari*	0.07049	0.10620	0.1084	0.08811

Nuclear loci. Networks of PRLR and UBN1 haplotypes (Fig. 2) congruently show that candidate species CS2-*subfulva* and CS3-Wbanded contain exclusive sets of haplotypes, none of which are shared with the other forms in either gene. The remaining three candidate species display sharing of some haplotypes. In both genes, one shared haplotype was found to be widespread across the range of CS1-*melanoleuca* and CS4-Wblack. In PRLR, the remaining CS4-Wblack specimens contained a near-exclusive haplotype, which was otherwise shared only by a single specimen of the Central African CS1-*melanoleuca* from Cameroon. The single specimen of *N. peroescobari* contained a unique, unshared haplotype in PRLR, but shared its single UBN1 haplotype with specimens of the Central African CS1-*melanoleuca* and the West African CS4-Wblack. CS2 displays considerable variation among its haplotypes. In PRLR, all specimens from the Indian Ocean coast sampled shared a unique, exclusive haplotype, whereas in UBN1, the coastal populations contained three unique and exclusive haplotypes, whereas other specimens shared a haplotype with other specimens from across the range of the species.

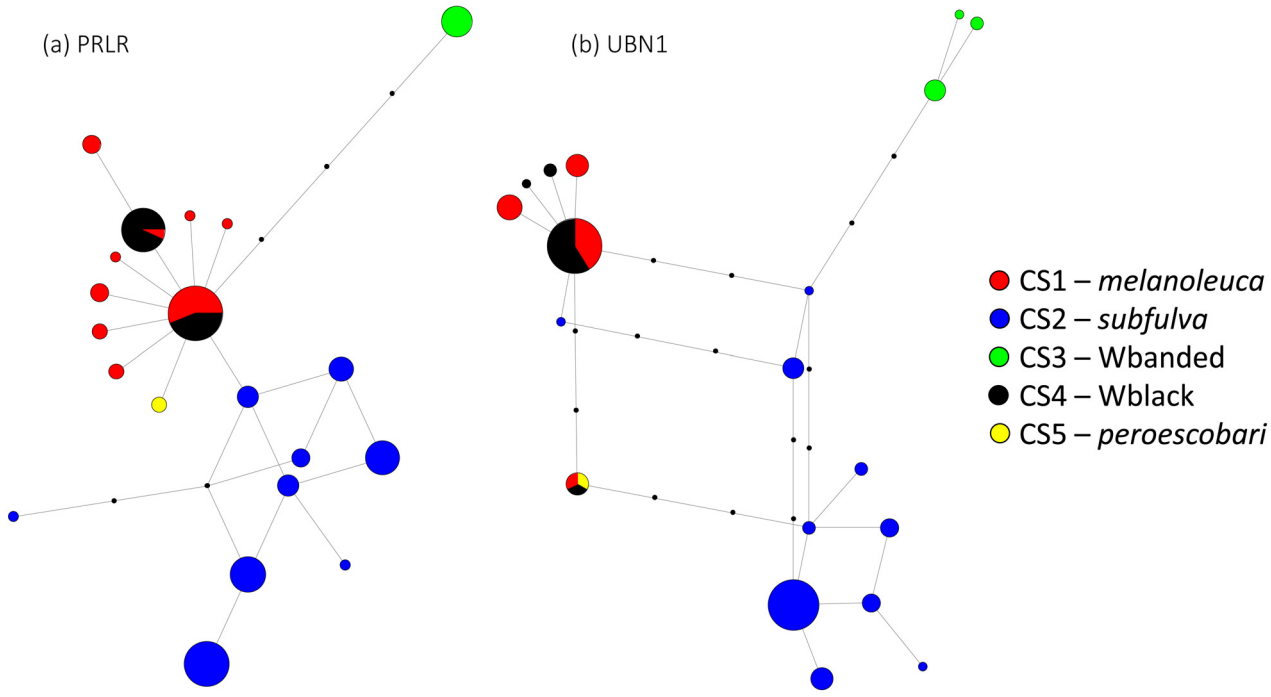

FIGURE 2. Haplotype networks for single copy nuclear loci. (a) PRLR; (b) UBN1. Small black circles indicate unsampled haplotypes.

The principal coordinates analysis (PCD) of standardised multilocus distances generated by POFAD (Fig. 3) shows clear separation of CS2-*subfulva* and CS3-Wbanded from each other and the remaining candidate species.

Reanalysis under exclusion of these highly distinct forms reveals partial separation between CS1-*melanoleuca*, CS4-Wblack and *N. peroescobari*, although some specimens of CS1-*melanoleuca* and CS4-Wblack share identical combinations of haplotypes.

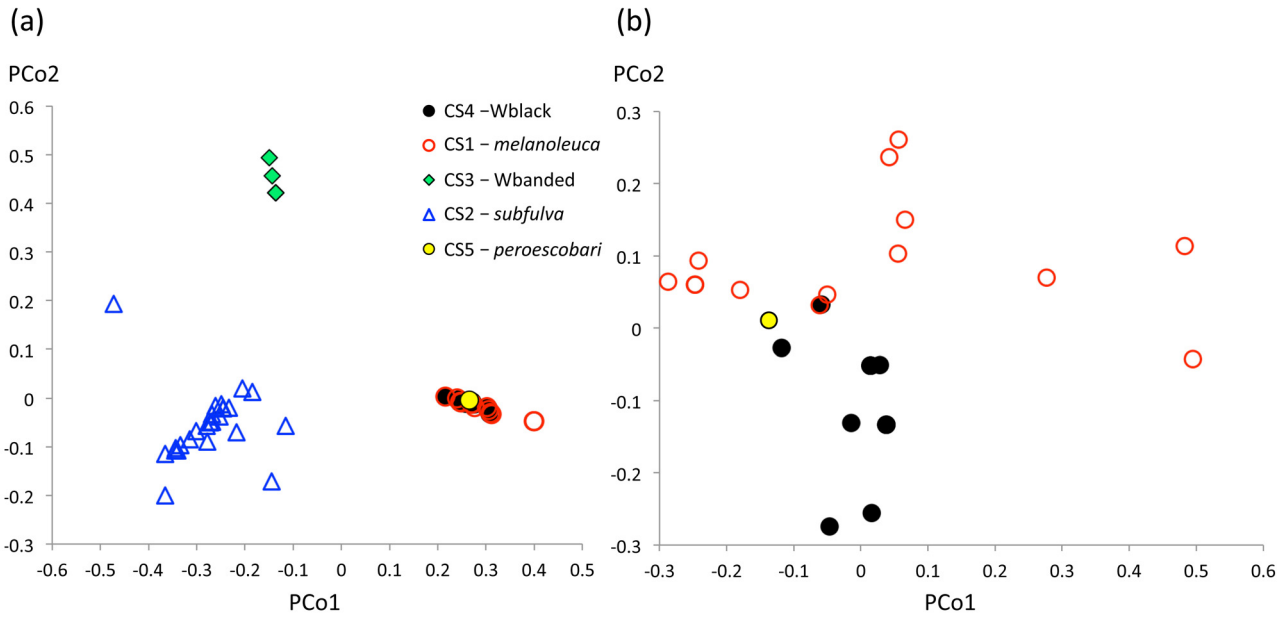

FIGURE 3. Ordination of individual specimens in a Principal Coordinates Analysis of standardised multilocus distances of PRLR and UBN1 scnDNA sequence data. (a) All specimens; (b) Analysis repeated under exclusion of CS2 and CS3.

TABLE 5. Candidate species and their posterior probabilities in BPP v3.

	Small ancestral population, shallow divergences	Large ancestral population, shallow divergences	Large ancestral population, deep divergences	Small ancestral population, deep divergences
Ancestral population size and divergence age priors	θ = 2 2000 τ = 2 2000	θ = 1 10 τ=2 2000	θ = 1 10 τ = 1 10	θ = 2 2000 τ = 1 10
CS1-*melanoleuca*	> 0.995	> 0.95	> 0.95	> 0.999
CS1-*melanoleuca* + CS5-*peroescobari*	0.0005–0.0012	0.043–0.050	0.022–0.044	< 0.0005
CS5-*peroescobari*	> 0.995	0.897–0.898	0.924–0.928	> 0.995
CS4-Wblack + CS5-*peroescobari*	0.0013–0.0025	0.060–0.0751	0.0281–0.0540	< 0.002
CS3-Wbanded + CS5-*peroescobari*	0	<0.0002	<0.0002	0
CS4-Wblack	>0.995	0.925–0.940	0.946–0.972	> 0.995
CS3-Wbanded	1	>0.9998	>0.9998	1
CS2-subfulva	1	1	1	1
Posterior probability for 4 species	0.002–.003	0.101–0.102	0.072–0.076	0.0005–0.0016
Posterior probability for 5 species	>0.997	0.898–0.899	0.924–0.928	0.9984–0.9995

Species delimitation analyses. The Bayesian species delimitation analyses using BPP3 supported the status of all five candidate species as distinct species under most priors of ancestral population size and divergence age (Table 5). The candidate species CS2-*subfulva* and CS3-Wbanded were consistently recognised as separate species. This applied irrespective of gamma parameters for θ and τ. CS1-*melanoleuca* (Central African forests) and CS4-Wblack (Upper Guinea forests) were also consistently recovered as separate species. CS5-*peroescobari* (São Tomé – *N. peroescobari*) was strongly supported as a separate species under reconstructions assuming small ancestral population sizes, but much more weakly under large ancestral population size scenarios, when dissenting trees were approximately evenly split between placing this population into conspecificity with either CS1-*melanoleuca* and CS4-Wblack. Nevertheless, both the latter remained supported as separate species irrespective of the position of CS5-*peroescobari*.

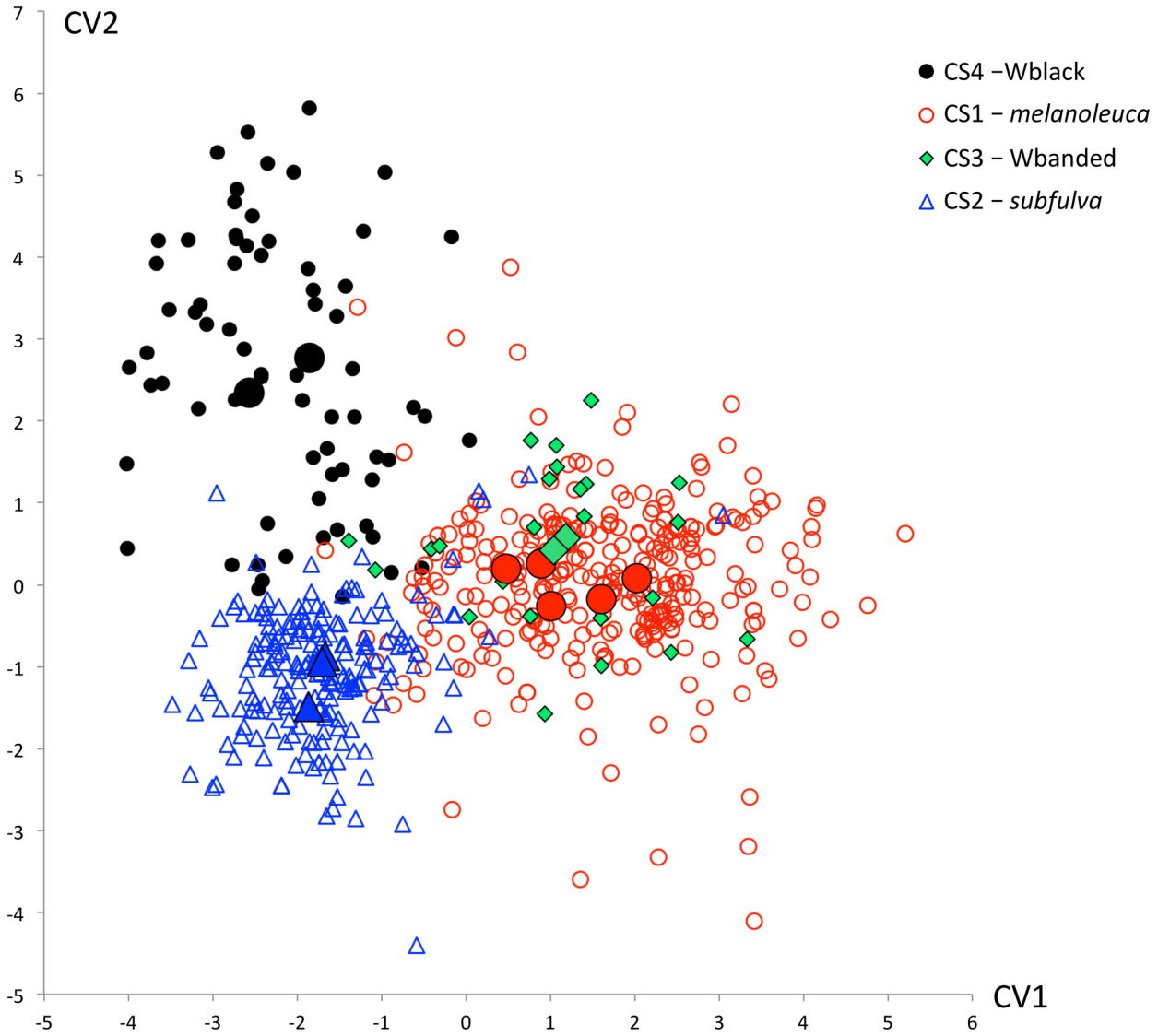

FIGURE 4. Ordination of individual specimens and OTU centroids of four of the mitochondrially defined candidate species of the *N. melanoleuca* complex along the first two canonical variates. CS5-*peroescobari* was omitted due to the small available sample size. Canonical variates 1 and 2 account for 57.9 and 22.8% of total variance, respectively. Enlarged symbols indicate OTU centroids.

Morphology. The morphological analyses revealed relatively subtle patterns of distinctness between the candidate species. Fig. 4 shows the ordination of individual specimens and OTU means of the canonical variates analysis of the morphological data of the full data of the five candidate species, and Table 6 shows the canonical variate loadings for the 8 characters retained in the analysis. The specimens representing the OTUs of CS1-*melanoleuca*, CS2-*subfulva* and CS4-Wblack are ordinated as largely distinct clusters with relatively little overlap,

whereas specimens of the less well sampled CS3-Wbanded are not separated from CS1-*melanoleuca*. Additional analyses (not shown) did not improve the resolution of CS3.

TABLE 6. Pooled within-group correlations between discriminating variables and standardized canonical discriminant functions. Character numbers follow Table 2.

	1st canonical variate	2nd canonical variate
1. Midbody scale rows	0.237	-0.698
2. Ventrals	0.471	0.146
3. Subcaudals	0.261	0.287
4. Number of main ventral bands	0.534	-0.266
5. Number of accessory ventral bands	0.203	0.115
7. First dark ventral of first main band	0.0173	0.603
9. Width of 1st main ventral band	-0.161	0.021
10. Last dark ventral of last discrete main band	0.773	-0.097

Taxonomy

In summary, the combined evidence from mitochondrial and nuclear DNA sequences and morphology support recognition of five mitochondrially defined candidate species as separate species. While not all species criteria support separate species status in all pairwise comparisons between candidate species, each pairwise comparison reveals one or several lines of evidence for the taxa involved. The evidence supporting pairwise species-level distinctness between the mitochondrially defined candidate species is summarised in Table 7.

TABLE 7. Distinguishing datasets and analyses supporting species status for the five mitochondrially defined candidate species considered in this study. Above the diagonal: evidence from nuclear loci; below the diagonal: evidence from morphology.

	CS1- *N. melanoleuca*	CS2- *N. subfulva*	CS3-Wbanded *N. savannula*	CS4-Wblack *N. guineensis*	CS5- *N. peroescobari*
CS1- *N. melanoleuca*	–	BPP POFAD no haplotype sharing in sympatry	BPP POFAD no haplotype sharing	BPP (POFAD)	BPP (POFAD)
CS2- *N. subfulva*	phenotypically distinct	–	BPP POFAD no haplotype sharing	BPP POFAD no haplotype sharing	BPP POFAD no haplotype sharing
CS3-Wbanded *N. savannula*	poorly distinct	phenotypically distinct	–	BPP POFAD no haplotype sharing in sympatry	BPP POFAD no haplotype sharing
CS4-Wblack *N. guineensis*	phenotypically distinct	phenotypically distinct	phenotypically distinct	–	BPP
CS5- *N. peroescobari*	diagnostic character states	diagnostic character states	diagnostic character states	diagnostic character states	–

The mitochondrial DNA analyses revealed deep haplotype clades with geographically cohesive distributions and partial range overlap. The levels of mtDNA sequence divergence between these candidate species equal or exceed those found between sister species of cobra in other subgenera of *Naja* (Slowinski & Wüster, 2000; Wüster

& Broadley, 2003, 2007; Trape *et al.*, 2009). These results are largely mirrored by the nuclear loci: species delimitation and POFAD analyses of the two loci analysed support the recognition of all five candidate species.

CS2-*subfulva* and CS3-Wbanded are unambiguously supported as distinct by a lack of haplotype sharing with any of the other candidate species, despite extensive sympatry with CS1-*melanoleuca* and CS4-Wblack respectively. This is reflected in unambiguous support for species status in BPP analyses and distinctness in POFAD ordinations. CS2-*subfulva* is additionally largely distinct from the other candidate species in the morphological analyses. We therefore recognise CS2-*subfulva* and CS3-Wbanded as distinct species.

CS1-*melanoleuca* and CS4-Wblack display haplotype sharing in both PRLR and UBN1. Such sharing of haplotypes can be the result of ongoing gene flow between the two forms, or the retention of ancestral haplotypes. These can be distinguished in part through the geographic distribution of haplotypes: retention of ancestral haplotypes predicts the distribution of the ancestral haplotype throughout the range of all taxa containing it, whereas gene flow predicts that shared haplotypes should be found primarily near the contact zone between the two taxa in at least one of them. In PRLR, the only extensively shared haplotype is found across the range of the Central African CS1-*melanoleuca* and the West African CS4-Wblack, from Guinea and Liberia to the Rift Valley, suggesting that this is a retained ancestral haplotype. Another common haplotype is nearly exclusive to CS4-Wblack, and the only specimen of CS1-*melanoleuca* that shares it originates from southwestern Cameroon, the westernmost population of CS1-*melanoleuca* included in the molecular study. In UBN1, an extensively shared haplotype is again found across the range of both CS1-*melanoleuca* and CS4-Wblack, suggesting that it represents a shared ancestral haplotype. Both CS1-*melanoleuca* and CS4-Wblack contain exclusive private haplotypes not shared with each other. The BPP analysis consistently categorised the Central and West African forest populations as separate species, and the morphological analyses also supported their distinctness. We therefore regard them as distinct species.

CS5-*peroescobari*: genetically, our single specimen of *N. peroescobari* is homozygous for a unique haplotype in PRLR, but shares a haplotype with a Ghanaian specimen of CS4-Wblack and two Congolese specimens of CS1-*melanoleuca* (*N. melanoleuca*) in UBN1. In BPP, support for species status of CS5-*peroescobari* was dependent on ancestral population size and root age, with more conservative parameter values resulting in reduced support for species status. Interestingly, the BPP iterations not recognising CS5-*peroescobari* as distinct were approximately equally split in associating it with either the CS1-*melanoleuca* or the CS4-Wblack, while strongly supporting the latter two as separate species. The species was described on the basis of the diagnostic character of separated posterior chin shields (not included in our analyses) and the restriction of white ventrals to the first 22 ventral scales. Our data contradict the latter character: several of our specimens from São Tomé have light ventrals extending further along the venter, to ventrals 47 (BMNH 1906.3.30.80) and even 56 (MBL 1954). Nevertheless, given the data presented in Ceríaco *et al.* (2017) and here, we recognise *Naja* (*Boulengerina*) *peroescobari* as an island endemic species restricted to São Tomé.

In terms of nomenclature, three of the five candidate species have available names. The Central African forest form (CS1) corresponds to *Naja melanoleuca* Hallowell, 1857, described from Gabon, and also includes *Naja haje* var. *leucosticta* Fischer, 1885. CS2-*subfulva* contains the holotype of *Naja melanoleuca subfulva* Laurent, 1955, described from Lwiro, South Kivu Province, Democratic Republic of Congo, and we therefore recognise it as a distinct species, *Naja* (*Boulengerina*) *subfulva*, which also includes the Cameroonian grassfields populations described as *Naja melanoleuca aurata* by Stucki-Stirn (1979). The name *Naja peroescobari* Ceríaco *et al.* 2017 is available for CS5.

In contrast, the two West African candidate species do not appear to have available names (we follow Kaiser *et al.*, 2013, Measey, 2013, and Kaiser, 2014 in not considering certain names published outside the peer-reviewed literature as part of the permanent scientific record), and we therefore describe them as new.

Naja (*Boulengerina*) *guineensis* sp. nov. Broadley, Trape, Chirio, Ineich & Wüster

Naia melanoleuca (not Hallowell) Boulenger, 1896: 376 (part, var. B [c,d], C).
Naja sp. 2 cf. *melanoleuca* Hallowell, 1857 (blackish dorsum) Trape & Baldé, 2014: 318.
Naja sp. 2 cf. *melanoleuca* (forest form). Trape & Baldé, 2014: 336.
Holotype: MNHN 1921.0485, a male from N'Zébéla, Macenta Prefecture, Nzérékoré region of forested southeastern Guinea (8° 05'N, 9° 05'W), elev. 490 m, Coll. Paul Chabanaud (1876–1959) between 1919–1920 (Chabanaud, 1921: 471) (Fig. 5).

Paratype: BMNH 1960.1.3.72, a male from Njala, Kori, Sierra Leone, Coll. C.T. Pyne.

Diagnosis. *Naja guineensis* can be distinguished from the partly sympatric *N. savannula* sp. nov. by lacking extended dorsal banding, often having 17 rather than 19 dorsal scale rows at midbody, a generally lower subcaudal scale count, fewer ventral bands, a lesser posterior extent of the ventral banding, and a strong tendency towards melanism in adults. Specimens with 19 midbody dorsal scale rows can be distinguished from *N. melanoleuca* through the reduced number of ventral bands, lesser posterior extent of banding and tendency of ontogenetic melanism from *N. subfulva* in lacking a lighter anterior dorsum and through ontogenetic melanism, and from *N. peroescobari* in having the posterior chin shields in contact.

FIGURE 5. *Naja* (*Boulengerina*) *guineensis* **sp. nov.** Left and top right: holotype, MNHN 1921.0485, dorsal and ventral view and side view of head. Note extensive mottling of throat and anterior ventral side and limited posterior extent of lighter ventral markings. Bottom right: live adult specimen measuring approximately 200 cm total length, from Sekondi-Takoradi, Western Region, Ghana, displaying dark suffusion of throat and anterior venter (not preserved; photo L. Chirio).

Description of holotype. Dimensions: A male specimen with dissected tail base, snout–vent length 1850 mm, tail length 370 mm, total length 2220 mm, ratio total length: tail length 6:1.

Body scalation: 23 scale rows around hood, 17 around midbody, 13 one head length ahead of the vent, all smooth and oblique; 208 ventrals, 62 subcaudals, all divided, anal single.

Head scalation: 7/7 supralabials, 3rd & 4th contact orbit, 6th largest; 8/8 infralabials, first 4 contact anterior chin shields; anterior pair of chin shields and infralabials 3–4 with an anomaly on both sides, they are warty and covered with circumvolutions; 1/1 preocular, twice as long as high; 3/3 postoculars; 1/1 anterior temporal; posterior temporals 3/3; rostral broader than high and slightly damaged anteriorly, clearly visible from above; 7 temporals and nuchals contacting both parietals.

Pattern: upper side of head and upper temporal region dark brown including parietals and temporals but black behind them, lips and ventral side of head creamy yellowish without dark mottling on chin and anterior throat; each posterior supralabial edge broadly outlined in black from eye level to mouth corner; temporals uniformly brownish. Dorsum uniform brownish-black throughout. Venter: anterior first 32 cm (14% of total length) of venter with

alternating yellow and blackish irregular bands, the posterior yellow bands increasingly suffused with dark mottling. Only ventral 1 is uniform clear yellow, ventrals 2–10, 19, 28–29, 32–35 yellow but always increasingly suffused with dark mottling, remaining ventrals and underside of tail black (after ventral 35).

Variation. Midbody dorsal scale row counts of 17 and 19 are approximately equally common in this species. In large adults, light pattern elements on the head and throat often become heavily suffused with black pigment, leading to a virtually entirely melanistic snake. Some specimens have 1–4 generally faint or poorly defined light bands across the neck, and occasionally an ocellate hood marking.

Largest recorded: 1818+437 = 2255 mm, from Ballassou, Guinea (IRD 4213.G), but larger specimens have been observed. Based on locality, Menzies' (1966) report of a specimen measuring "eight feet, eight inches" (264 cm) from Bo, Sierra Leone, is likely to refer to this species.

Etymology. The specific epithet *guineensis* means "from Guinea" and is chosen to reflect the distribution of the species in the Upper Guinea forests of West Africa, part of the West African Forests biodiversity hotspot (Myers *et al.*, 2000).

Suggested common name. Black forest cobra.

Distribution. The distribution of *Naja guineensis* appears to be restricted to the Upper Guinea Forests of western Africa, from western Togo to Liberia and Guinea (Trape & Baldé, 2014) (Fig. 6). There is a single record from Contuboel, Guinea Bissau (MBL 535). All other records 10°N or lower.

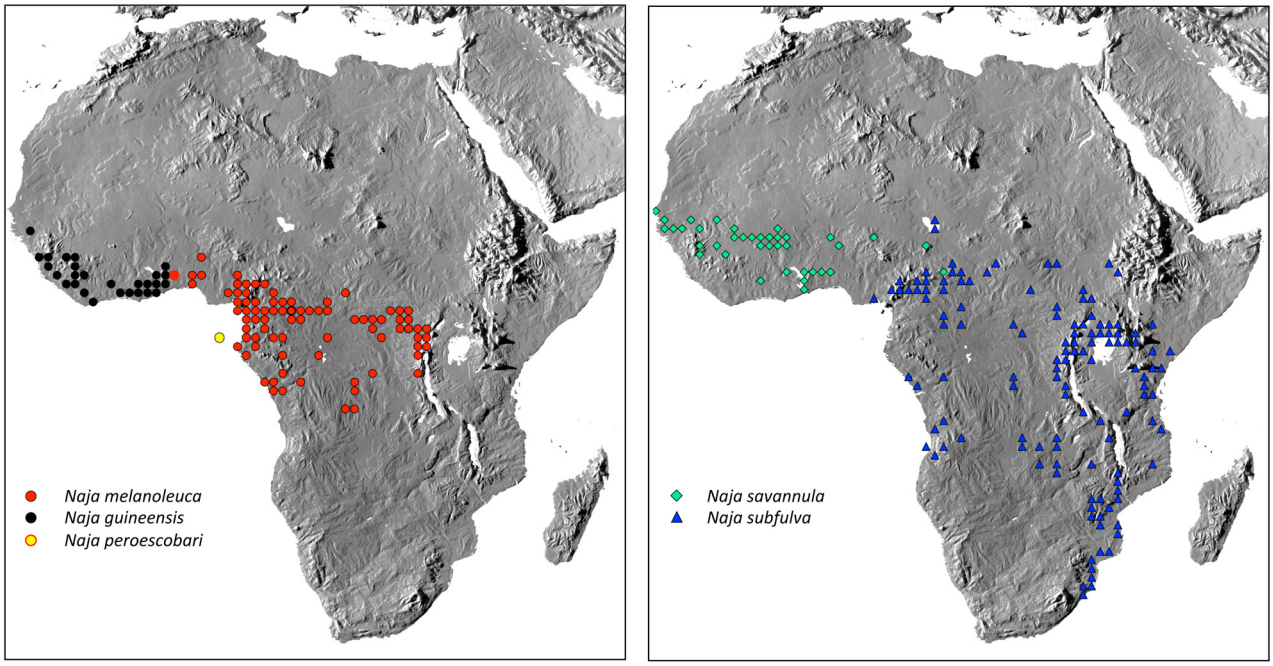

FIGURE 6. Distribution of the five species of the *N. melanoleuca* complex. A full list of voucher specimens beyond those included in multivariate analyses and Appendix 2 is available on request from the first author.

Naja (*Boulengerina*) *savannula* sp. nov. Broadley, Trape, Chirio & Wüster

Naia melanoleuca (not Hallowell) Boulenger, 1896: 376 (part, var. D).
Naja "banded form" Hughes, 2013: 128.
Naja sp. 1 cf. *melanoleuca* Hallowell, 1857 (yellow banded dorsum) Trape & Baldé, 2014:318.
Naja sp. 1 cf. *melanoleuca* (banded savanna form) Trape & Baldé, 2014: 336.
Holotype: MNHN 2018.0002, an adult male collected by Laurent Chirio on 31/03/2007 in Niénié, W Biosphere Reserve, Benin, 11.05920 °N, 2.20488 °E, elevation 272 m (Fig. 7).
Paratype: BMNH 1975.657, a male specimen collected on the grounds of Amadou Bello University Hospital, Zaria, Nigeria, by D.A. Warrell.

Diagnosis: Distinguishable from other species of the *N. melanoleuca* complex by the presence of 3 to 8 semidivided yellowish or whitish bands on the anterior dorsal forebody, becoming uniform black caudad; venter yellow with 2 to 8 black bands. Generally higher mean subcaudal scale counts than the other species. Genetically diagnosable through possession of unique mitochondrial haplotypes (cytochrome *b*: GenBank MH337597–602; ND4: MH337403–408) and unique PRLR and UBN1 haplotypes (PRLR: MH337501–504; UBN1: MH337532–535).

Description of holotype. Dimensions: A male specimen, snout–vent length 1815 mm, tail length 376 mm, total length 2191 mm, ratio total length: tail length 5.8.

Body scalation: 25 scale rows around hood, 19 around midbody, 15 one head length ahead of the vent, all smooth and oblique. Dorsal scales smooth, oblique. Vertebral row not enlarged. 213 ventrals (and one incomplete ventral before the anal), 69 subcaudals, all divided, anal single.

Head scalation: 7/7 supralabials, 3rd & 4th contact orbit, 6th largest; 8/8 infralabials, first 4 contact anterior chin shields; posterior chin shields in narrow contact at their anterior end, separated by median scale along most of their length; 1/1 preocular, twice as long as high; 3/3 postoculars; 1/1 anterior temporal; 3/3 posterior temporals; rostral broader than high, clearly visible from above; 7 temporals and nuchals contacting the parietals.

Pattern: upper side of head brown, lips and ventral side of the head pale creamy yellowish with each labial scale laterally bordered with black. Neck brown, dorsum becoming gradually black towards rear. Two very distinct pale dorsal crossbands at the level of ventrals (V) 24 to 28, two other at level of V 40–45, three at level of V 58–64, three less distinct at level of V 77–83, and three slightly marked at level of V 98–104. Ventrals 1 to 11, 13–19, 23–31, 38–47 and 57–66 pale creamy yellowish, V 20–22 and 32–37 black; V 12 and 48–56 yellowish spotted with black spots. From ventral 67 to the vent, ventrals spotted with black gradually become completely black. Subcaudals black.

Variation: Dorsal scale rows on neck 19–25, at midbody 19, before vent 12–15; ventrals 211–233, subcaudals 63–77 (Table 8). Dorsal semi-divided yellow bands 3–8; ventral principal black bands 2–8 (Fig. 7).

FIGURE 7. *Naja* (*Boulengerina*) *savannula* **sp. nov.** Top row and bottom left: holotype, MNHN 2018.0002. Bottom right: live specimen from Kindia, Guinea, showing conspicuous, broad dorsal bands and ventral banding, including narrow accessory bands (not vouchered). Photos J.-F. Trape.

Etymology: The name is derived from the contraction of its savanna habitat and annulated colour pattern and was coined by Barry Hughes in an unpublished 1968 manuscript. We have retained this name at the request of our colleague Barry Hughes.

Largest recorded: 1825+405 = 2230 mm, from Medina Djikoye, Senegal (IRD 6155.S).

Suggested common name: West African banded cobra.

Distribution: Senegal and Gambia east to northern Cameroon (Fig. 6). *Naja savannula* appears to be restricted to gallery forest areas in Africa in Guinean Forest/Savanna Mosaic, extending northwards into West Sudanian Savanna (Chirio, 2003, 2013; Monasterio *et al.*, 2016). Our records are mostly from latitudes 10–14°N, except in the Dahomey Gap in eastern Ghana and Benin, where the species approaches the Gulf of Guinea Coast. The eastern extent of the range is poorly understood. A specimen from Margui Wandala district, northern Cameroon (approx. 10.5°N 13.6°E; MNHN 1962.0022) appears to be assignable to this species, and one of us (JFT) recently collected a specimen from Mboura, 20 km SW Baïbokoum, Logone Oriental Province, Chad (7.598°N, 15.596°E; IRD 2281.N), and there is a recent record from near Niamey, Niger (LC, unpublished data). It seems likely that the species has a wider distribution in northern Cameroon, extreme southern Chad and possibly even extreme northwestern Central African Republic (CAR).

Naja (*Boulengerina*) *melanoleuca* Hallowell, 1857

Naia haie var. *melanoleuca* Hallowell, 1857, *Proc. Acad. Nat. Sci. Philadelphia*: 61. Type locality: Gabon, syntypes ANSP 6875–76, 6878–79.

Naja haje var. leucosticta Fischer, 1885, *Jahr. Hamburg. Wiss. Anst.* **2**: 115, pl. v, fig. 11. Type locality: Cameroon and Ogooué River, Gabon, syntypes ZMH 4280, 7048, 7299–7302.

Naia melanoleuca; Boulenger, 1896: 376 (part, vars. A [a,b] & B [a,b])

Naja melanoleuca melanoleuca; Laurent, 1956: 290, pl. xxvi, fig. 2.

Naja (*Boulengerina*) *melanoleuca;* Wallach *et al.*, 2009.

Boulengerina melanoleuca; Wallach *et al.*, 2014: 122.

Aspidelaps bocagei Sauvage, 1884: 204 (type locality: Gabon and Majumba; holotype MNHN 1884.0015) has been listed as a synonym of *N. melanoleuca* (e.g., Broadley, 1983; Wallach *et al.*, 2014; Ceríaco *et al.*, 2017), but is in fact a synonym of *Naja annulata*, as is evident from Sauvage's description, which notes approximately 20 dark double bands along the entire body length, and 21 mid-dorsal scale rows (see also Schmidt, 1923).

Diagnosis: Dorsum black, often with 1–3 semidivided yellow crossbands on the neck, the first may be an ocellus; venter yellow with 4 to 6 black bands in the first 100 ventrals, thereafter uniform black.

Variation: Dorsal scale rows on neck 19–27, at midbody 19 (very rarely 17 or 21); ventrals 209–230; subcaudals 59–74 (Table 8). Supralabials 7, the third and fourth entering the orbit; infralabials 8, the first four in contact with the anterior sublinguals, no cuneate; preocular 1; postoculars 3 (very rarely 2 or 4); temporals 1+2 or 1+3; nuchals bordering temporals 5–9, usually 7.

FIGURE 8. *Naja* (*Boulengerina*) *melanoleuca.* Adult specimens from Yaoundé, Cameroon (left—photo J.-F. Trape) and Tsibilé, Gabon (right—photo L. Chirio). Note the diffuse but distinct hood mark that is often present in this species, and the combination of broad main bands and narrow accessory bands on the ventral side.

TABLE 8. Variation in meristic characters in the species of the *N. melanoleuca* complex.

	Naja guineensis *N*=71		*Naja melanoleuca* *N*=307		*Naja savannula* *N*=24		*Naja subfulva* (excl. eastern and southern African pops.) *N*=202		*Naja peroescobari* *N* = 8	
	Mean ± 1SD	Range	Mean ± 1SD	Range	Mean ± 1SD	Range	Mean ± 1SD	Range	Mean ± 1SD	Range
Midbody dorsal scale rows	17.0 ± 1.0	17–19	19.0 ± 0.3	17–21	19.0 ± 0	19	19.0 ± 0.2	18–21	19.0 ± 0.0	19
Ventrals	211.8 ± 4.2	203–221	217.8 ± 4.4	206–232	216.9 ± 4.2	211–226	210.0 ± 4.8	197–228	209.4 ± 3.4	204–215
Subcaudals	65.1 ± 2.3	60–70	65.5 ± 2.7	57–74	70.0 ± 1.6	67–73	62.8 ± 2.8	57–72	66.2 ± 4.6	57–70
First dark ventral of first main band	18.0 ± 2.3	10–23	16.3 ± 1.7	11–21	16.4 ± 2.2	11–21	14.2 ± 1.7	10–22	18.2 ± 3.4	14–22
Last dark ventral of first main band	22.2 ± 2.3	16.26	19.9 ± 1.9	15–25	19.4 ± 2.4	14–25	18.2 ± 2.1	13–26	22.63 ± 6.4	15–29
Width of first main band (in ventral scales)	5.2 ± 1.4	2–9	4.5 ± 1.0	2–9	4.0 ± 0.8	3–5	5.0 ± 1.0	2–10	5.4 ± 3.1	2–9
Number of main bands	1.5 ± 0.7	1–4	3.7 ± 0.9	1–7	3.6 ± 1.3	1–7	2.5 ± 0.8	1–5	1.7 ± 0.7	1–3
Number of accessory bands	0.8 ± 1.2	0–5	1.4 ± 1.7	0–9	1.4 ± 1.9	0–6	0.3 ± 0.79	0–4	0.7 ± 0.9	0–2
Total number of discrete dark bands	2.3 ± 1.4	1–6	5.1 ± 1.7	2–12	5.0 ± 2.5	2–12	2.8 ± 1.1	1–8	2.4 ± 1.2	1–5
Last ventral of last discrete dark band	28.5 ± 9.2	17–52	68.6 ± 15.4	24–114	59.4 ± 19.1	19–103	36.8 ± 10.0	13–82	27.6 ± 15.4	15–55

Colouration: Head brown, the supralabials barred black and yellow, chin yellow. Black above, sometimes a yellow monocellate marking on the hood, or 1–3 small yellow blotches, the dorsal scales may be tipped with white in juveniles (Fig. 8). Yellow or white below with 4 to 6 black bands on the first 100 ventrals, usually uniform black thereafter.

Largest recorded: 2250+420 = 2670 mm, from Moniya, Ibadan, Nigeria (Butler, 1982: 110).

Suggested common name: Central African forest cobra.

Distribution: Centered on the Congo Basin, west to southwestern Nigeria and possibly southern Benin, south to northern Angola, not extending east of the Albertine Rift Valley, where it is replaced by *N. subfulva.* The western range limits are poorly understood. Populations from southeastern Nigeria are clearly assignable to this form. A few specimens from Lamta, southern Benin (IRD 12.B, IRD 54.B, IRD 60.B), and Ghana (MNHN 1983.0663–64; no further locality information) also appear to be assignable to *N. melanoleuca*.

Naja (*Boulengerina*) *subfulva* Laurent, 1955

Naia melanoleuca (not Hallowell); Boulenger, 1896: 376 (part, vars A [c] & E).

Naja melanoleuca subfulva Laurent, 1955, Revue Zool.– Bot. Afr. 51: 132. Type locality: Lwiro, 1850 m, Kabare, Kivu, Belgian Congo [= Democratic Republic of Congo], holotype MRAC 17514.

Naja melanoleuca aurata Stucki-Stirn, 1979, Snake Report 721: 617–620. Type locality: Bamenda, Cameroon. No type designated.

Naja subfulva; Chirio & Ineich, 2006: 54.

Naja melanoleuca subfulva; Chirio & LeBreton, 2007: 584.

Naja (*Boulengerina*) *melanoleuca subfulva;* Broadley & Blaylock, 2013: 139.

Naja (*Boulengerina*) *melanoleuca*; Conradie *et al.,* 2016: 28.

Diagnosis. Midbody scale rows 19, except along coastal regions of East Africa (Kenya, Tanzania), where most specimens have 17 rows. Pattern highly variable. Adults of most populations distinguishable in having a brown forebody, often with spots, generally becoming darker or blackish posteriorly. Labial pattern may be attenuated in many adults. Venter with several black, dark brown or greyish crossbands on the first 50 ventrals, gradually becoming uniform black caudad in some populations, but often remaining entirely light, often with extensive darker spotting or speckling. Where present, the light forebody and/or light posterior venter are diagnostic for this species. Generally fewer ventral bands and ventral scales than *N. melanoleuca* or *N. savannula* and fewer subcaudals than *N. savannula* (Table 8). Genetically diagnosable through possession of unique mitochondrial haplotypes (cyt *b*: GenBank MH337603–633; ND4: MH337409–439) and unique PRLR and UBN1 haplotypes (PRLR: MH337441–471; UBN1: MH337531, MH337536–562, MH337564–566).

Variation. Dorsal scale rows on neck 19–27, at midbody 19 (very rarely 17 or 21); ventrals 196–226; anal entire; subcaudals 55–71 (Table 8). Supralabials 7 (very rarely 5 or 6), the third and fourth entering orbit; infralabials 8 (rarely 7), the first four (rarely three) in contact with the anterior sublinguals, no cuneate (very rarely one); preocular 1; postoculars 3; temporal 1+2 or 1+3; temporal bordering parietals 5–9, usually 7.

Colouration. Head brown, the supralabials usually barred black and yellow, chin yellow. Dorsum uniform black, or with faint white crossbars or white-tipped dorsal scales, resulting in a stippling effect, in all juveniles. Adults from the periphery of Lake Victoria and parts of the Congo Basin (Fig. 9) have a uniformly black dorsum as adults, whereas in most of the range, adults become brown anteriorly. There may be 2 or 3 faint yellow divided crossbars on the neck. Ventral banding often faint in adults, with grey or pale brown ventral bands that may be very inconspicuous, or lacking altogether. Narrow accessory ventral bands usually absent.

Largest recorded. 2016+380 = 2396 mm (tail truncated) [JPT 1856 – Zinave, Mozambique], but NMZB-UM 17594 from Inyangani Tea Estates, Zimbabwe, had a total length of 2690 mm (only head and neck preserved).

Suggested common name. Brown forest cobra. This suggestion reflects the fact that in most populations, with the exception of the surroundings of Lake Victoria and some locations in the Congo Basin, the anterior body of adults is brown rather than black. We note that the distribution of this species includes many non-forested habitats, including savannas and open wetlands (Spawls *et al.*, 2018). However, retention of the word "forest" in the common name conveys the phylogenetic information that this species is part of the *Naja melanoleuca* complex.

Distribution. Forest/savanna mosaic, encircling the Congo Basin, from the grassfields of western Cameroon northward, reaching 13.45°N at Bol, Chad, on the northern shore of Lake Chad (IRD 2802.N), east through the

CAR and South Sudan to western Ethiopia, south through Uganda, western Kenya, eastern DRC, Rwanda, Burundi and western Tanzania to northern Malawi, west through Zambia and Katanga to Angola and the Lower Congo region (Fig. 6). The distribution extends disjunctly east and south to the East African coast from Kenya to northern KwaZulu-Natal (South Africa), including inland locations in western Zimbabwe. The precise distribution of this taxon in the Congo region remains to be ascertained: we have here provided molecular evidence of multiple specimens of this species from the evergreen forest zone of northern Republic of Congo (Likouala Forest – Jackson *et al.*, 2007) and the northeastern DRC (northwestern Orientale Province along the Congo River). Some of these specimens and populations are difficult to differentiate from sympatric *N. melanoleuca*, necessitating the use of DNA barcoding approaches (Hebert *et al.*, 2003; Clause *et al.*, 2016) to ensure reliable identification. Two specimens from southeastern Nigeria with low ventral scale counts and lacking ventral banding or displaying a single faded band may also be assignable to this species (Degema, Rivers State: BMNH 1902.11.10.9, and "Oil River": BMNH 88.8.29.20), suggesting a wider distribution in eastern Nigeria.

Comment. This species displays considerable geographic variation in pattern and scalation, as well as indications of phylogeographic structure. The present study focused on the relationships among the five mitochondrially defined candidate species, and did not encompass the full range of morphological variation in *N. subfulva*. It is possible that future work may identify additional cryptic diversity within this wide-ranging and variable species.

FIGURE 9. *Naja* (*Boulengerina*) *subfulva*. Variation in colour and pattern. Top left: specimen from Kakamega, western Kenya, illustrating the typically deep black and white specimens with strong facial markings from the periphery of Lake Victoria. Bottom left: specimen from Chuka, Mount Kenya, Kenya, illustrating an extreme of the brown forebody and reduced facial pigmentation typical of the species in much of its range. Photos W. Wüster, courtesy Royjan Taylor / Bio-Ken snake farm live collection, Watamu, Kenya. Right: specimen from Bamenda, Cameroon, representing the form described by Stucki-Stirn (1979) as *Naja melanoleuca aurata*. Note the indistinct ventral bands and the lack of accessory ventral bands, as is typical of this species. Photo J.-F. Trape.

Naja (*Boulengerina*) *peroescobari* Ceríaco *et al.*, 2017

Diagnosis. In the original description, *Naja peroescobari* was diagnosed from the other members of the *N. melanoleuca* group through a lack of white ventrals posterior to ventral 22, a lack of lighter markings on the

dorsum, and the separation of the posterior chin shields. Our sample does not fully support the diagnostic value of these characters: at least one specimen (BMNH 1906.3.30.80) has the posterior chin shields in contact, and discrete dark bands separated by lighter bands (although often suffused with dark brown pigment) extend as far back as ventrals 45 and 55, respectively, in BMNH 1906.3.30.80 and MBL 1954. *Naja peroescobari* is distinct from *N. subfulva* in never having a brown forebody or a light posterior venter and in lacking dark speckling or spotting on the forebody. It displays greatly reduced ventral banding compared to *N. melanoleuca* and *N. savannula*, and, unlike *N. guineensis*, never has 17 midbody dorsal scale rows.

The species is also diagnosable through unique mitochondrial haplotypes (Ceríaco *et al.*, 2017; cyt *b*: GenBank MH337634; ND4: MH337440) and a unique PRLR haplotype (MH337499)

Variation. See Table 8 and Ceríaco *et al.* (2017) for variation in scale counts and ventral banding in this species.

Distribution. Restricted to the volcanic island of São Tomé in the Gulf of Guinea, where it seems to inhabit primarily the forested parts of the centre and south of the island, whereas it seems to be missing from the more open northeast (Ceríaco *et al.*, 2017).

Discussion

The results of this study illustrate the level to which we still remain ignorant of the species diversity of even some of the most iconic organisms alive today. Forest cobras are common in captive collections of venomous snakes in much of the world, and they are one of the most common groups of snakes in many parts of their distribution. Nevertheless, little effort had been made until now to investigate the status of the various populations of the complex.

Some of this confusion is due to subtle patterns of morphological variation in the complex. Broadley (1968) noted the inconsistency between scalation and pattern when assessing the status of Laurent's *N. m. subfulva* when it came to Ugandan and western Kenyan specimens. Moreover, while the distinction between species may be reasonably obvious in some places, it may not be so in others: Laurent (1955), Stucki-Stirn (1979) and Chirio & Ineich (2006) had little difficulty in separating *N. melanoleuca* and *N. subfulva* in eastern DRC, Cameroon and CAR, whereas one of us (Jackson *et al.*, 2007) collected both species from sites approximately 80 km apart in the Likouala Forest of the northern Republic of the Congo, and remained unaware of the heterogeneity of the samples prior to the genetic results: the specimens involved are morphologically virtually indistinguishable, with the local *N. subfulva* (from Ganganya Brousse) displaying a pattern virtually identical to that usually associated with *N. melanoleuca* as found by Jackson *et al.* (2007) at their second site, Impongui.

Morphologically virtually indistinguishable cryptic species are common in some groups (see Bickford *et al.*, 2007, for a review), but have not been reported commonly among larger snakes: even though species delimitation in many groups has required molecular and multivariate morphometric approaches, the recognised species, once delimited, were normally diagnosable without undue difficulty (e.g., Slowinski & Wüster, 2000; Broadley & Wüster, 2004; Wüster & Broadley, 2003, 2007; Trape *et al.*, 2009; but see Malhotra *et al.*, 2011b; Mrinalini *et al.*, 2015). The *N. melanoleuca* group may be unusual in this regard. We acknowledge, however, that our morphological data were limited in terms of numbers of usable characters, and the use of multiple sources in the compilation of the data may have introduced unintended imprecision into the data presented here, as well as making the assignment of some specimens to species difficult. Rigorous and thorough studies of the external and internal morphology of this complex may yet reveal reliable diagnostic character states for the different species.

The intraspecific variability, lack of clear morphological differentiation and occurrence in macrogeographic sympatry (with, more than likely, parapatry on a smaller geographical scale—Laurent, 1955; Stucki-Stirn, 1979) of several species of the complex complicates the diagnosis of species, the identification of some specimens, and the establishment of the distribution of the species of the complex. A combined morphological and molecular barcoding approach would be helpful to confirm the morphological diagnosis and distribution of the species of this complex, especially around the Dahomey Gap and adjoining parts of Nigeria, Cameroon and southern Chad, where all four mainland species approach each other. In particular, while all southern Togolese material has been assignable to *N. guineensi*s, a series of specimens from Lamta, Benin, appears to be assignable to *N. melanoleuca*. Further east, the distributions of *N. melanoleuca*, *N. savannula* and *N. subfulva* appear to approach each other or

overlap in northern Cameroon. Specimen IRD 2281.N, from Mboura, Chad (see above) was assignable to *N. savannula*, whereas another from Moundou, 100 km to the NE (8.57°N, 16.07°E; IRD 2600.N), was assignable to *N. subfulva*. The distribution and diagnosis of *N. melanoleuca* and *N. subfulva* in the Congo Basin also remain insufficiently resolved. Because Central Africa is one of the most poorly sampled regions in Africa by herpetologists for a variety of reasons (Tolley *et al.*, 2016; Greenbaum, 2017), extensive fieldwork efforts will be required to fill in gaps in sampling and improve understanding of the geographic distribution of these cobras.

The taxonomic division of widespread species raises the possibility that some of the newly recognised species may be much more threatened than previously suspected (e.g., Melzer *et al.*, 2017; Shaney *et al.*, 2017): *Naja melanoleuca* in its original sense has never been assessed for its IUCN Red List status, but its wide distribution and common occurrence in many areas suggests that it is unlikely to be highly threatened as a whole. This may not be true of some of the newly recognised species with much more restricted distributions. As indicated by the common name, all the species of this complex are primarily snakes of wooded or forested habitats, although *N. savannula* and *N. subfulva* also occupy gallery forests and wetlands in savanna habitats (Trape & Mané, 2006; Marais & Jubber, 2010). Although, like most cobras, forest cobras adapt well to moderate anthropogenic changes (Menzies, 1966), there is evidence that they are prone to being replaced by *N. nigricollis* in deforested habitats in at least some parts of their range (Luiselli & Angelici, 2002), suggesting that they are likely to be vulnerable to the effects of forest loss, and their exploitation for bushmeat has also been documented (Eniang *et al.*, 2006). This would be a particular concern for *N. guineensis*, which is restricted to the Upper Guinean Forest sector of the West African Forests Global Biodiversity Hotspot (Myers *et al.*, 2000). Equally, *N. peroescobari* is restricted to the island of São Tomé, a WWF-designated priority ecoregion (Jones, 1994; WWF & IUCN, 1994), and the species is potentially vulnerable due to its isolated insular range as well as persecution out of fear, or for traditional Chinese medicine (Ceríaco *et al.*, 2017).

The hitherto unsuspected diversity of the *N. melanoleuca* complex revealed in this study also suggests that a reassessment of venom composition and its variation in the complex is required, with a particular view towards testing the efficacy of the available antivenoms against the different venoms of the complex. This group has been largely neglected in the recent toxinological literature, but a recently published proteomic study (Lauridsen *et al.*, 2017) used venom from Ugandan specimens, suggesting that they represent *N. subfulva.* Most prior papers on the venoms of this complex failed to provide locality indications, precluding identification to species level, and potentially leading to problems of repeatability of previous work (Wüster & McCarthy, 1996).

Our more refined understanding of the systematics of the complex also provides a more robust underpinning for further work on the evolution of venom composition in the ecologically diverse subgenus *Boulengerina.* This includes a mixture of small and large terrestrial, burrowing and aquatic forms occupying a diversity of biomes and with distinct foraging ecologies (Spawls & Branch, 1995; Spawls *et al.*, 2004), providing a promising system for testing hypotheses of adaptation in venom composition (Barlow *et al.*, 2009). In addition, it should direct ecological studies in regions of potential sympatry between forest cobra species, to determine any difference in activity, habitat and dietary preferences, etc., that may yield fuller understanding of these remarkable African snakes.

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APPENDIX 1. Specimens used for molecular analyses. Specimens without museum vouchers are either tissues only or in private collections. Museum collection acronyms follow Leviton *et al.*, 1985. WW = Wolfgang Wüster tissue archive; IRD = Institut de Recherche pour le Développement, Hann, Dakar; CRT = Congo River Trip 2010 field number, specimens to be accessioned in IRSNB; USNM-FS = USNM field series, specimen not vouchered. 1 = sequenced.

Sample number / voucher	Taxon, candidate species	Locality/origin	cyt *b*	ND4	PRLR	UBN1
WW580	*Naja naja*	Sri Lanka	1	1		
WW1430 - NMK S/3993	*Naja ashei*	Watamu, Kenya	1	1		
WW837	*Naja nubiae*	unknown, London Zoo live collection	1	1		
MNHN 2008.0074	*Naja senegalensis*	Dielmo, Senegal	1	1		
WW1084 – Latoxan, live collection N. me. me. 20001.	CS4-Wblack	Nkawkaw, Ghana	1	1	1	1
WW1658 – MVZ 249816	CS4-Wblack	Nkwanta, Volta, Ghana	1	1	1	1
WW2456 – IRD 90.T	CS4-Wblack	Togo	1	1	1	1
WW2491 – IRD 364.T	CS4-Wblack	Togo	1	1	1	1
WW2492 – IRD 366.T	CS4-Wblack	Togo	1	1	1	1
WW2493 – IRD 369.T	CS4-Wblack	Togo	1	1		
WW3749 – Laurent Chirio 8237X	CS4-Wblack	Bokoume, Guinea	1	1	1	1
WW3751 Laurent Chirio 8274X	CS4-Wblack	Gbanwoe, Guinea	1	1	1	1
WW3752 – Laurent Chirio 8357X	CS4-Wblack	Gbanshue, Liberia	1	1	1	1
WW3753 – Laurent Chirio 8452X	CS4-Wblack	Bokoume, Guinea	1	1	1	1
WW3754 – Laurent Chirio 8478X	CS4-Wblack	Gbamou, Guinea			1	1
WW3757 – Laurent Chirio 8720X	CS4-Wblack	Gbanshue, Liberia	1	1	1	1
WW3758 – Laurent Chirio 8765X	CS4-Wblack	Bunadin, Liberia	1	1	1	1
WW3759 – Laurent Chirio 8796X	CS4-Wblack	Yai, Guinea	1	1	1	1
WW182	CS1-*melanoleuca*	Nyasoso, Cameroon	1	1	1	1
WW1877	CS1-*melanoleuca*	Lobeke National Park, Cameroon	1	1	1	1
WW1878	CS1-*melanoleuca*	Lobeke National Park, Cameroon	1	1	1	1
WW1879	CS1-*melanoleuca*	Lobeke National Park, Cameroon	1	1	1	

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APPENDIX 1. (Continued)

Sample number / voucher	Taxon, candidate species	Locality/origin	cyt *b*	ND4	PRLR	UBN1
WW2719 – USNM-FS 246560	CS1-*melanoleuca*	Impongui, Likouala Region, RoC	1	1		
WW2720 – USNM 558272	CS1-*melanoleuca*	Impongui, Likouala Region, RoC	1	1	1	1
WW2721 – USNM 558271	CS1-*melanoleuca*	Impongui, Likouala Region, RoC	1	1	1	1
WW2722 – USNM 558273	CS1-*melanoleuca*	Impongui, Likouala Region, RoC	1	1	1	1
WW2996 – USNM 576163	CS1-*melanoleuca*	Impongui, Likouala Region, RoC	1	1	1	1
WW2873 – PEM R16698	CS1-melanoleuca	Ayol Alar, Ogooue-Ivindo Province, Gabon			1	1
WW3163 PEM R20904	CS1-*melanoleuca*	Soyo, Zaire Province, Angola	1	1	1	1
WW3797 – ELI 499 – UTEP 20348	CS1- *melanoleuca*	Bizombo, South Kivu, DRC	1	1	1	1
CRT3779	CS1-*melanoleuca*	Kona, Orientale, DRC	1	1	1	1
CRT3913	CS1-*melanoleuca*	Bomane, Orientale, DRC	1	1	1	1
CRT4044	CS1-*melanoleuca*	Bomane, Orientale, DRC	1	1	1	1
WW1197 – CAS 219403	CS5-*peroescobari*	São Tomé	1	1	1	1
WW1085 – Latoxan live collection N. me. me. 20002.	CS3-Wbanded	Nkawkaw, Ghana	1	1	1	1
WW1539 – TR563	CS3-Wbanded	Friguiagbe, Guinea	1	1	1	1
WW2046 – Laurent Chirio 6588	CS3-Wbanded	Nienie, Benin	1	1		
WW2047 – Laurent Chirio 6589	CS3-Wbanded	Nienie, Benin	1	1		
WW2495 – IRD 8490.S	CS3-Wbanded	Keur Sen Gueye, Siné-Saloum, Senegal	1	1	1	1
WW3755 – Laurent Chirio 8626X	CS3-Wbanded	Founoukouroudou, Guinea	1	1	1	1
WW1086 – Latoxan live collection N. me. su. 97340001	CS2-*subfulva*	Bamenda, Cameroon	1	1	1	1
WW1087 – Latoxan live collection N. me. su. 98050002	CS2-*subfulva*	Bamenda, Cameroon	1	1	1	1
WW1088 – Latoxan live collection N. me. su. 98050003.	CS2-*subfulva*	Bamenda, Cameroon	1	1	1	1
WW1090. Vivarium d'Yvoire, live collection	CS2-*subfulva*	Burundi	1	1	1	1
WW1649 – Latoxan live collection – 0112-6	CS2-*subfulva*	Bamenda, Cameroon	1	1	1	1
WW1089 – Ophiofarm, Servion, live collection.	CS2-*subfulva*	Bangui, CAR	1	1	1	1

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APPENDIX 1. (Continued)

Sample number / voucher	Taxon, candidate species	Locality/origin	cyt *b*	ND4	PRLR	UBN1
WW1587	CS2-*subfulva*	Ngotto, CAR	1	1	1	1
WW1912 – MNHN 2006.0530	CS2-*subfulva*	Mboki, CAR	1	1	1	1
WW2723 – USNM 576076	CS2-*subfulva*	Ganganya Brousse, Likouala Region, RoC	1	1	1	1
WW2997 – USNM-FS 246312	CS2-*subfulva*	Ganganya Brousse, Likouala Region, RoC	1	1	1	1
CRT4004	CS2-*subfulva*	Bomane, Orientale, DRC	1	1	1	1
CRT4056	CS2-*subfulva*	Bomane, Orientale, DRC	1	1	1	1
CRT3585	CS2-*subfulva*	Yaekela, Orientale, DRC	1	1	1	1
CRT3690	CS2-*subfulva*	Yaekela, Orientale, DRC	1	1	1	1
EBG 1126 - UTEP 20349	CS2-*subfulva*	Mbayo, South Kivu, DRC	1	1	1	1
ELI 406 (no voucher)	CS2-*subfulva*	Idjwi Island, Lake Kivu, DRC	1	1	1	1
EBG 2198 – UTEP 20350	CS2-*subfulva*	Muchoga, Maniema, DRC	1	1	1	1
EBG 2278 (no voucher)	CS2-*subfulva*	Sange, Luvungi, South Kivu, DRC	1	1	1	1
WW2876 – Colin Tilbury 622	CS2-*subfulva*	Uganda	1	1		
WW1264 – Bio-Ken live collection BK10044	CS2-*subfulva*	Kakamega, Kenya	1	1	1	1
ELI 29 – UTEP 20351	CS2-*subfulva*	Pweto, Katanga, DRC	1	1	1	1
WW1265 – Bio-Ken live collection BK10153	CS2-*subfulva*	Chuka, Kenya	1	1	1	1
WW4009 – Bio-Ken live collection BK12395	CS2-*subfulva*	Chuka, Kenya	1	1	1	1
WW1266 – Bio-Ken live collection BK10046	CS2-*subfulva*	Arabuko-Sokoke, Kenya	1	1	1	1
WW4006 – Bio-Ken live collection BK10803	CS2-*subfulva*	Arabuko-Sokoke, Kenya	1	1	1	1
WW4007 – Bio-Ken live collection BK11452	CS2-*subfulva*	Arabuko-Sokoke, Kenya	1	1	1	1
WW4008 – Bio-Ken live collection BK11511	CS2-*subfulva*	Arabuko-Sokoke, Kenya	1	1	1	1
WW189 – PEM R 15498	CS2-*subfulva*	Marromeu, Mozambique	1	1	1	1
WW1292	CS2-*subfulva*	Hluhluwe, KwaZulu-Natal	1	1	1	1
WW1326	CS2-*subfulva*	Hluhluwe, KwaZulu-Natal	1	1	1	1
WW2654	CS2-*subfulva*	Cape Vidal, KwaZulu-Natal	1	1	1	1

APPENDIX 2. Material examined for morphological analyses. Bold type indicates specimens included in molecular analyses. Collection acronyms follow Leviton *et al.* (1985), except: BH = Barry Hughes, personal collection; IRD = Institut de Recherche pour le Développement, Hann, Dakar, Senegal; LC = Laurent Chirio, personal collection.

Naja melanoleuca: **ANGOLA**: MBL 1950. **BENIN.** IRD 12.B, IRD 54.B, IRD 60.B. **CAMEROON**. AMNH 51816–20; BH E3M108; BMNH 96.5.27.2; BMNH 1906.5.28.20; BMNH 1937.12.1.101; BMNH 1957.1.13.94; BMNH 1967.152–155; BMNH 1968.49–50; BMNH 1968.51a; BMNH 1971.409; CAS 16956; CM 15186; CM 15189–90; CM 15195; CM 15200; CM 15203; CM 15205; CM 15221; CM/S 7210; CM/S 9233; CM/S 9268; CM/S 9310; CM/S 9316; CM/S 9328; FMNH 19465-66; FMNH 58959; Hughes E3M108; MCZ 7859a–c; MCZ 10071; MCZ 13216; MCZ 22842; MNHN 1958.0072; MNHN 1988.2466; MNHN 2005.3483–84; MNHN 2005.3520; MNHN 2005.3529; MNHN 2005.3532; MNHN 2005.3538; MNHN 2005.3550; MNHN 2005.3553; MNHN 2005.3559; MNHN 2005.3561; MNHN 2005.3575–76; MNHN 2005.3585; MNHN 2005.3619; MNHN 2005.3622; MNHN 2005.3646; MNHN 2005.3650; MNHN 2005.3652–54; MNHN 2005.3659–60; MNHN 2005.3678; MNHN 2005.3686; MNHN 2005.3703–04; MNHN 2005.3708–09; MNHN 2005.3721; MNHN 2005.3730; MNHN 2005.3743; MNHN 2005.3750–51; MNHN 2005.3753; MNHN 2005.3756; MNHN 2005.3771; MNHN 2005.3774–75; MNHN 2005.3777; MNHN 2005.3780–82; MNHN 2005.3789; MNHN 2005.3793–94; NMZB-UM 2668; SMF 104; UMMZ 35580; UMMZ 38843–44; ZMB 14716; ZMB 18521–24; ZMB 18526; ZMB 20244; ZMB 20263–65; ZMB 20272; ZMB 20371; ZMB 20379; ZMB 20699; ZMB 26793; ZMB 27607; ZMB 28056; ZMB 30871; ZMB 31555; ZMB 32042; ZMUC 6508; LC 0125M; LC 0361X; LC 0866I; LC 0918I; LC 0941C; LC 1066C; LC 1109C; LC 1281X; LC 1282X; LC 1399X; LC 1860X; LC 2930X; LC 2960X; LC 3028X; LC 3066X; LC 3938X; LC 3995X; LC 4465I; LC 4805X; LC 4840X. **CENTRAL AFRICAN REPUBLIC**. AMNH 120491; MCZ 55409; MNHN 1963.0891; MNHN 1964.0519; MNHN 1968.0212; MNHN 1985.0094; MNHN 1992.4391; MNHN 1992.4541; MNHN 1992.4544; MNHN 1994.8371–72; MNHN 1994.8374; MNHN 1995.3697–98; MNHN 1995.3700; MNHN 1995.3711–12; MNHN 1996.7010; MNHN 1996.7012; MNHN 1996.7017; MNHN 1996.7022–27; MNHN 1996.7031–32; MNHN 1996.7034; MNHN 1996.7037; MNHN 1999.9334. **REPUBLIC OF CONGO (Brazzaville)**. MNHN 1965.0388–89; MNHN 1966.0758; MNHN 1966.0762; MNHN 1971.0385; MNHN 1987.1573; MNHN 1987.1575–79; MNHN 1987.1585–87; MNHN 1987.1589; MNHN 1987.1592–95; MNHN 1987.1597; MNHN 1987.1699; USNM 558272–73; USNM 576163; ZMUC 65525; ZMUC 65526; ZMUC 65527; ZMUC 65528. **DEMOCRATIC REPUBLIC OF CONGO**. AMNH 12320-22; AMNH 12324; AMNH 12368-70; AMNH 12372-73; AMNH 12375-78; ANSP 20746; BMNH 1901.3.12.103; BMNH 1919.8.16.97; BMNH 1930.6.11.54; BMNH 1979.213; CM 69366; IRSNB 3858; IRSNB 8559; LACM 49560; LACM 49581; MCZ 24746; MCZ 25957-58; MCZ 53996; NMZB 3739; PEM 3405; PEM 13982; RGMC 1661; RGMC 10510; RGMC 15832; RGMC 20579; RGMC 21672; RGMC 3254; RGMC 4210; RGMC 5167; UMMZ 172965; USNM 142593–4; USNM 167040-44; USNM 216229; UTEP 20348; UTEP 21784**;** ZMB 20224a. **EQUATORIAL GUINEA**. AMNH 16935–6; ZMUC 6517. **GABON**. ; ANSP 27251; ANSP 6875-76; ANSP 6878-79; BMNH 86.5.15.33–34; CAS 16981; FMNH 75032; MBL 1951; MCZ 51910; MNHN 1885.0721–22; MNHN 1896.0555–56; MNHN 1899.0192–93; MNHN 1966.0759–61; MNHN 1967.0464–67; MNHN 1967.0470–71; MNHN 1967.0473–76; USNM 62110; USNM 62147; ZMB 6297; ZMUC 65310. **NIGERIA**. BH E3M83; BMNH 1948.1.2.87; BMNH 1971.410; BMNH 88.8.29.19; CM 92606; CM 92691; CM 92714; CM 92715; CM 92716; Hughes E3M83; MCZ 6325; UMMZ 61250; USNM 157076; ZMUC 6526-29; ZMUC 6539.

Naja guineensis: **CÔTE D'IVOIRE**. LACM 108940; MNHN 1885.0706; MNHN 1957.0084; MNHN 1973.0437; MNHN A816; MNHN 1977.0565; MNHN A816. **GHANA**. MNHN 1983.0663–64; BH E3M28; BH E3M32; BH E3M43; BH E3M46; BH E3M52; BH E3M54-58; BH E3M63; BH E3M76; BH E3M82; BMNH 1946.3.20.18; BMNH 1960.1.5.43; CAS 103277; FMNH 44382; FMNH 44431; FMNH 44433; FMNH 44435–6; FMNH 53648; MCZ 53739; MCZ 55280–1; USNM 223939; ZMB 2809; ZMC 65506. **GUINEA**. MNHN 1899.0282; MNHN 1921.0485 (holotype); MNHN 1951.0161; MNHN 1962.0402–03. **LIBERIA**. MNHN 1986.1625; MNHN 1986.1810; MNHN 1986.1849–50; MNHN 1990.4599; FMNH 118084; FMNH 178905–06; FMNH 178908; FMNH 179351; FMNH 191428; FMNH 191430–32; FMNH 58116–17; FMNH 58120–21; MCZ 51815; MCZ 5709; MNHN 1986.1702; ZMUC 65312. **SIERRA LEONE**. BMNH 1958.1.1.51; BMNH 1960.1.3.72; FMNH 121968. **TOGO**. IRD 14.T; IRD364.T; IRD366.T; IRD369.T; USNM 223904–606; ZMB 26662; ZMB 6908.

Naja peroescobari: **SÃO TOMÉ E PRÍNCIPE**. BMNH 1906.3.30.80; MBL 1954; MBL 1960; MHNG 2518.041; MHNG 2522.064; MSNG 31022a-b; MSNG 37595.

Naja savannula: **BENIN**. IRD 12B; IRD 54B; IRD 60B; IRD 77B; USNM 199604. **CAMEROON**. MNHN 1962.0022; MNHN A733. **CÔTE D'IVOIRE**. MNHN 1992.4284. **GAMBIA**. BMNH 56.11.15.3. **GHANA**. BH E3M104; BH E3M25; BH E3M36; BH E3M79; CAS 154799; MHNG 2030–68. **GUINEA**. IRD 79.G; IRD 93.G; IRD 119.G; IRD 159.G; IRD 305.G; IRD 373.G; IRD 429.G; IRD 491.G; IRD 583.G; IRD 1188.G; IRD 1253.G; IRD 1459.G; IRD 1786.G; IRD 1868.G; IRD 2344.G; IRD 3069.G; IRD 3313.G; IRD 3438.G; IRD 3491.G; IRD 3518.G; IRD 3573.G; IRD 3968.G; IRD 4901.G; IRD 4988.G. **MALI**. IRD 245.M; IRD 767.M; IRD 995.M; IRD 1782.M; IRD 2027.M; IRD 2048.M; IRD 2087.M; IRD 2101.M; IRD 3716.M; MNHN 1921.0618. **NIGERIA**. BMNH 1975.657. **SENEGAL**. IRD 1731.S; IRD 1884.S; IRD 2129.S; IRD 3431.S; IRD 5163.S; IRD 5165.S; IRD 5502.S; IRD 5522.S; IRD 6155.S; IRD 6240.S; IRD 6469.S; IRD 8112.S; IRD 8317.S; IRD 8334.S; IRD 8387.S; IRD 8395.S; IRD 8490.S; ZFMK 17578; BMNH 1968.606. **TOGO**. IRD 192.T; CAS 136118; ZMB 20269.

Naja subfulva: BURUNDI. UTEP 21780. CAMEROON. MNHN 2005.3485; MNHN 2005.3495; MNHN 2005.3518; MNHN 2005.3522; MNHN 2005.3544–45; MNHN 2005.3551–52; MNHN 2005.3562; MNHN 2005.3589–90; MNHN 2005.3596; MNHN 2005.3624; MNHN 2005.3627–28; MNHN 2005.3634; MNHN 2005.3641; MNHN 2005.3670; MNHN 2005.3675; MNHN 2005.3685; MNHN 2005.3689; MNHN 2005.3692–93; MNHN 2005.3698; MNHN 2005.3738; MNHN 2005.3752; MNHN 2005.3783; NMZB-UM 9818; CAS 104584; MVZ 191877; LC 1419C; LC 3016X; LC 3295I; LC 3414I; LC 4496I. **CENTRAL AFRICAN REPUBLIC**. MCZ 55409; MNHN 1964.0515; MNHN 1964.0517; MNHN 1991.0312; MNHN 1992.4540; MNHN 1992.4542; MNHN 1994.8359–61; MNHN 1994.8363; MNHN 1994.8367–68; MNHN 1994.8370; MNHN 1995.3694–96; MNHN 1995.3706–09; MNHN 1996.7038; MNHN 1999.8738; MNHN 1999.8743; MNHN 1999.8799; MNHN 1999.9136. **REPUBLIC OF CONGO**. USNM 576076; MNHN 1988.2437. **DEMOCRATIC REPUBLIC OF CONGO**. AMNH 12315; RGMC 3997; RGMC 4278; RGMC 4286; RGMC 6010; RGMC 12228; RGMC 16382; RGMC 16531; RGMC 17514; RGMC 17520; RGMC 17521; RGMC 17526; RGMC 17558-59; RGMC 76-3-R-428; RGMC 76-3-R-442; UTEP20349; UTEP 20351; UTEP 21781; UTEP 21782; UTEP 21783. **ETHIOPIA**. BMNH 1976.1669. **KENYA**. CAS 101519; CAS 122749; LACM 21607; MCZ 40730–40; NMK 53; NMK 1157; NMK 1199; NMK 1486–8; NMK 1555b; NMK 2355a-b; NMK 2406; NMK 2713; NMK 2753; NMK 2857; NMK 2940; CAS 152793. **SUDAN**. FMNH 48084; FMNH 62218–19; MCZ 53498; ZMUC 6512. **UGANDA**. ANSP 20781; BMNH 94.2.10.10; BMNH 1901.2.12.7; BMNH 1901.6.24.58; BMNH 1909.3.29.11; BMNH 1929.8.5.25; BMNH 1930.6.11.55; BMNH 1934.12.15.602; BMNH 1951.1.5.35; BMNH 1951.1.5.37; BMNH 1954.1.12.49; BMNH 1954.1.12.50; BMNH 1954.1.12.51a–c; BMNH 1959.1.7.38–66; BMNH 1959.1.7.68–69; BMNH 1959.1.7.72-74; BMNH 1960.1.2.39; BMNH 1976.2270; CAS-SU 21713; CAS-SU 21715; CM 38169; LACM 48306; LACM 48315; LIVCM 1962.330.5; LIVCM 1962.9.5; MCZ 30407–9; MCZ 40725–28; MCZ 47807; MCZ 47834; MCZ 48463–66; MUZM (Pitman R1822); MUZM (Pitman R3157); MUZM 43; NMK 1607; NMZB 5481–2; UMMZ 88515.